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SUBJECT OF INVESTIGATION

EXPLORATION OF NEW CHEMOTHERAPEUTICS FOR INFECTIOUS DISEASES

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EXPLORATION OF NEW CHEMOTHERAPEUTICS

FOR

INFECTIOUS DISEASES

Fundamental Studies on Protomycin, an Antiamoebic Antibiotic and Cephalomycin, an Antiviral antibiotic

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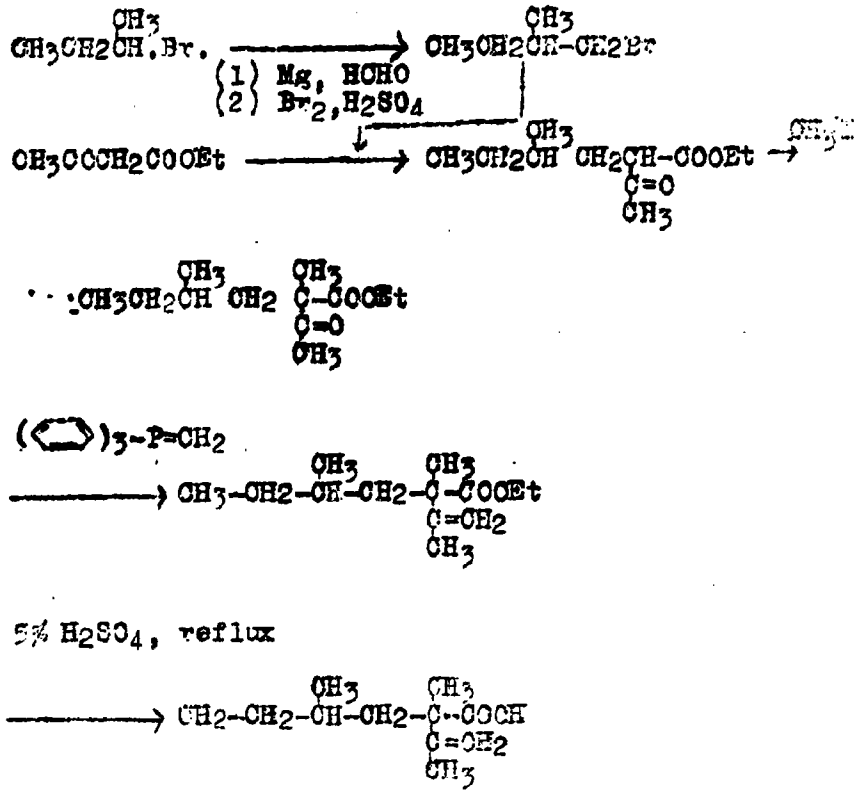
CHEMICAL STRUCTURE OF PROTOMYCIN

Tetrahydroprotomycin (I) was converted into oxime with hydroxylamine-pyridine in ethanol. The reaction mixture remained viscous inspite of several procedures of purification, so that it was treated with 10ml of 75% H_2SO_4 on boiling water bath to induce Beckmann's rearrangment. The reaction mixture was diluted with 20 volumes of water and steam distiled. The oily acid in the distillate was treated with p-phenylazophenacylbromide. The resulting ester (II) orange colored and melting at 54-55°C, satisfied the molecular formula, $C_{25}H_{30}N_2O_4$. Thus, the oxime of tetrahydro protomycin gave an acid, $C_{10}H_{19}COOH$, by Beckmann's rearrangment followed by hydrolysis.

The structure of the acid was proved by the synthesis of the acid through following steps, employing Wittig's reaction:



The synthetic acid was converted to p-phenylazophenacylester(IV) M.P. 60-62°C.

The mixed melting point (55°C), elemental analysis and infrared spectrum were sufficient to coclude the identity of the compounds (II) and (III).

A C_{11}-acid from tetrahydroprotomycin oxime.

A mixture of tetrahydroprotomycin (1.28g, 3.66 mol), pyridine (290cc) and hydroxylamine hydrochloride (254mg) in 10 ml ethanol was refluxed for two hours, evaporated, washed with water and ether and dried.

Since the residue remained viscous in spite of several treatments, 10 ml. of 80% sulfuric acid was added, heated on boiling water bath for 1 hour, poured into 200 ml. of cold water, and steam-distilled. The distillate containing floating oily material consumed 2.04 mol equivalent of sodium hydroxide on neutralization with standarized 0.1N NaOH. The neutralized solution was evaporated in vacuo and refluxed with 500 mg of p-phenylazophenacyl bromide in 80% aqueous ethanol for two hours. The reaction mixture was evaporated to dryness and separated from original reagent by silicic acid chromatography developed with benzene. Twice recrystalization from 95% EtoH gave orange needles (II), M.P. 54-55°C.

Anal. Calcd. for $C_{25}H_{30}N_2O_3$: C, 73.71; H, 7.79; N, 6.60.

Found: C, 73.86; H, 7.44; N, 6.99.

The elemental analysis suggested the original acid to have an empirical formula of $C_{10}H_{19-21}COOH$.

P-phenylazophenacyl-α-methyl,γ-iso-propenyl-β-methyl caproate (III)

Synthetic active amyl bromide (b.p. 121-125°C) for starting material was prepared according to conventional method of Grignard reaction and bromination from sec. butylbromide and formaldehyde, followed by treatment with H_2SO_4-HBr.

To 40 ml. of ethanol was dissolved 3g of metallic sodium and 17g of ethyl acetoacetate. The active amyl bromide prepared above (21.5g, 0.13 mol.) was added dropwisely to this solution with stirring over a period of 2 hours. After completion of the addition, the reaction mixture was heated to refluxing and stirred for more five hours, cooled, added with about 5 ml. of acetic acid and extracted with ether.

After removing ether, the residue was distilled in vacuo to obtain 9.5g (0.055 mol.) of ethyl active amyl-acetoacetate. B.P. 106°C, 10 mm. Hg.

To ethanol (25cc), dissolving 1.15g of metallic sodium was added the above obtained ethyl active amyl-aceto acetate. To the resulting solution was added methyl iodide (2.6g) dropwisly with stirring over a period of one hour and refluxed for additional three hours. The reaction mixture was treated as above. The distillate at 55°C (7 mm Hg) was collected. Yield 5.5g (0.025 mol.).

To 4.4g of bromobenzene in 14 ml. of ether was added 400 mg. of metallic lithium. After spontaneous reaction had subsided, the reaction mixture was heated to reflux to dissolve remaining portion of the metal. To the resulting solution was added 12g of triphenyl methyl phosphonium bromide and stirred for 30 min. The above prepared ester (5.5g) in 13 ml. ether was added and stired for 30 minutes, followed by refuxing for five hours. The resulting mixture was filtered and the filtrate was evaporated. The residue was distilled to give an oily material 43-45°C (7 mm Hg), 1.2g (0.006 mol.).

The oily material was refuxed with 200 ml. of 5% H_2SO_4 for five hours and steam distilled. The distillate was neutralized with standardized 0.1[illegible]N (0.0038 mol.), dried up in vacuo and treated with p-phenylazophenacyl bromic in ethanol. The resulting ester (III) was freed from the reagent through silicic acid chromatography and recrystallized from ethanol. Chromatography on silicic and recrystallization were repeated, in refering to infrared spectrum.

The infrared spectrum of the ester was completely identical with the corresponding derivative from protomycin (II) Mixed melting point of (II) and (III) was 550°C in several ratios of mixing.